DECLARATION

I, Ursula Scherz of Schlesierstr. 8 in 81669 München, Federal Republic of Germany, do hereby declare that I am conversant with the English and German languages and am a competent translator thereof. I declare further that the following is a true and correct translation into English made by me of the original text of the German patent application DE 102 01 862.6.

Signed this 16th day
of July 2007

Ursula Scherz

K 3125

Conjugate for Treating Prokaryotic Infections

The present invention relates to a conjugate for treating prokaryotic infections from a transport mediator penetrating the prokaryotic cell membrane and a desired compound to be introduced into the prokaryote and directed thereagainst, which is preferably a peptide nucleic acid (PNA) directed against a prokaryote gene giving antibiotic resistance.

Bacterial infections which on account of a resistance formation can no longer be treated with the currently available antibiotics are advancing worldwide. The situation is critical above all in clinics, e.g. in intensive-care units, where antibiotics are administered daily and pathogens can easily develop resistances. Also, the incorrect or excessive use of antibiotics has rapidly increased the number of resistant bacterial strains. Although the problem has previously been solved by using alternative or newly developed antibiotics (e.g. cephalosporins/derivatized antibiotics) and combination therapies (e.g. cotrimoxazole/sulfonamides), new resistances also occur in this case in a relatively short period of time. For example, vancomycin, which was active against certain bacterial strains a short time ago, often can no longer be used in therapy on account of the meanwhile formed resistances. Although major efforts are made to develop new natural antibiotics of different origin from prokaryotes and eukaryotes, only temporary or partial success has been achieved with respect to the rapidly mutating bacterial strains.

Thus, the invention is substantially based on the technical problem of providing means enabling an effective therapy of diseases correlated with prokaryotes, e.g. bacteria, resistant to antibiotics.

This technical problem is solved by providing the embodiments characterized in the claims.

In order to solve this technical problem, the inventors re-sensitized the bacteria with respect to the (classical or modern) antibiotics against which they were resistant. For this sensitization a conjugate was developed comprising the following components: (a) a transport mediator for the prokaryotic cell membrane, *i.e.* preferably a peptide (e.g. defensin) antibacterial *per se*, which can penetrate, and damage, the bacterial cell wall on account of its charge and structure by pore formation, and (b) the compound to be introduced into the prokaryote, e.g. a peptide nucleic acid (PNA) which is preferably directed against a gene giving the prokaryote an antibiotic resistance. By means of the examples resulting in the present invention it was possible to show that the antibiotic resistance of bacteria (*E. coli*) resistant to ampicillin/neomyin, which were treated with specific defensin-conjugate-$PNAs_{ampicillin/neomycin}$, has been overcome successfully. Thus, the conjugates according to the invention are a new class of antibiotics which can overcome the bacterial defense mechanisms or by means of which it is possible to give back the original activity to already known classical antibiotics, such as benzylpenicillins, tetracyclines, and neomycins. This differs completely from the former therapy and research strategies, yet supplements them in a very promising way. The (dual) strategy which can be carried out by means of the present invention specifically addresses to prokaryotic, e.g. bacterial, membranes and is toxic to prokaryotes, on the one hand, and compounds directed against prokaryotes, e.g. antisense nucleic acids (anti-gene nucleic acids) directed specifically against bacterial genes, e.g. PNAs, peptide domains, modified nucleotides, inhibitors of enzymes, *etc.*, can be introduced by means of the conjugates according to the invention, on the other hand.

The conjugates according to the invention thus have *inter alia* the following advantages: (a) no expensive screening method is required for establishing them, and (b) the revitalization of classical antibiotics can be achieved in a special embodiment by blocking the prokaryotic resistance mechanisms on the nucleic acid level so that the "old" antibiotics which were no longer usable in many cases on account of their lacking activity, regain significance.

Thus, the present invention relates to a conjugate suited fro treating prokaryotic infections and comprising the following components:

(a) a transport mediator passing through the prokaryotic cell membrane; and
(b) a compound to be introduced into the prokaryote and directed thereagainst.

The term "compound to be introduced into the prokaryote and directed thereagainst" as used herein relates to any compound detrimental to the prokaryote, e.g. kills it or prevents the growth and/or division thereof. Suitable compounds are known to the person skilled in the art, they comprise e.g. anti-metabolites, modified nucleotides, *etc.*

As to methods of producing the individual conjugate components and their linkage, reference is made to German patent application No. 199 33 492.7 and the below examples. The other components (e.g. spacer and/or PNA) are linked to the transport mediator by a covalent chemical bond. Where appropriate, a redox cleavage site (-S⌒S-) between transport mediator and compound to be introduced can be introduced chemically by means of a redox coupling. Also, a covalent bond, preferably an acid-amide bond, exists between an optionally present spacer and the compound to be introduced, e.g. the PNA. Possible alternatives are ether or ester bonds, depending on the functional group(s) present in the compound to be conjugated.

The conjugate according to the invention is suited for treating all prokaryotic infections, e.g. infections with bacteria, preferably human pathogen bacteria, mycoplasms or yeasts.

The transport mediator of the conjugate according to the invention is preferably a peptide or protein which can penetrate the prokaryotic cell membrane and can introduce the desired compound into the cytoplasm. The length of this peptide or protein is not subject to limitation as long as it has the above property. The selected transport mediator is preferably produced biologically (purification of natural transport mediator proteins/peptide or cloning and expression of the sequence in a eukaryotic or prokaryotic expression system) and in particular

synthetically, e.g. according to the Merrifield method (Merrifield, J. Am. Chem. Soc. 85 (1963), 2149).

This transport mediator is preferably a compound which as such is already detrimental to the prokaryote, e.g. by damaging the membranes (e.g. by forming pores or lesions). These are preferably defensins or holins (bacteriophage protein domains).

In a particularly preferred embodiment, the conjugate according to the invention comprises a transport mediator which includes a phage-holin protein, a phage-holin protein with one of the amino acid sequences shown in figure 3 or fragments or variants thereof which can still penetrate the prokaryotic cell membrane, being even more preferred.

The terms "variant" and "fragment" used in the present invention comprise proteins/peptides having amino acid sequences which differ with respect to the sequences indicated in figure 3 by deletion(s), insertion(s), substitution(s) of amino acid residues and/or other modifications known in the art or comprise a fragment of the original protein/peptide, the variants and/or fragments of the protein/peptide substantially having maintained the biological properties of the initial protein/peptide, *i.e.* may penetrate the prokaryotic cell membrane. Methods of producing the above modifications in the amino acid sequence are known to the person skilled in the art and described in standard works of molecular biology, e.g. in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY (1989)). The person skilled in the art can also determine whether such a protein or peptide still has the desired biological properties, e.g. by the methods described in the below examples.

In an alternative preferred embodiment of the conjugate according to the invention, the transport mediator comprises a defensin, preferably a human defensin. The defensins are polypeptides or peptides having an antimicrobial activity, which represent important factors in the innate immunity of vertebrates and non-vertebrates. Defensins have been isolated from animals (including

humans), plants and insects, for example. They usually consist of 29 to 42 amino acids and contain three disulfide bridges formed of three cysteine pairs. Defensins suited for the present invention are described in Tang *et al.*, Science 286 (1999), 498; Saido-Sakanaka *et al.*, Biochem. J. 338 (1999), 29; Raj *et al.*, Biochem J. 347 (2000), 633; Yu et al., J. Biol. Chem. 275 (No. 6) (2000), 3943, for example.

In an even more preferred embodiment of the conjugate according to the invention, the compound to be introduced into the prokaryote is a peptide nucleic acid (PNA) directed against the expression of a gene. On account of their physicochemical properties, the use of the protease and nuclease-resistant peptide nucleic acids ("PNAs") which are oligonucleotide derivatives where the sugar phosphate backbone is preferably substituted by ethyl-amine-bound α–amino-ethyl-glycine units, permits a stable and efficient blocking of the transcription of the desired genes under physiological conditions. An anti-gene strategy based on the antisense-principle is pursued by using these PNAs, where it is not the mRNA but the gene *per se* that is the target. Here, the PNAs hybridize to the target DNA by forming a triple helix. The target area can be a transcribed region of the gene to be blocked, e.g. of the gene giving antibiotic resistance, on the one hand, or a regulatory region whose blocking by means of PNAs also inhibits the transcription, on the other hand. Suitable regions can be identified by the person skilled in the art by means of the known DNA sequences or the function thereof. The peptide nucleic acids preferably have a length of at least 15 base pairs, peptide-nucleic acids having a length of at least 18 bases are particularly preferred, and of at least 21 bases are even more preferred and of at least 23 base pairs are most preferred. The peptide nucleic acid can optionally be labeled, e.g. radioactively, using a dye, biotin/avidine *etc.* The synthesis of PNAs is known to the person skilled in the art and also described in Nielsen *et al.*, Science 254 (1991), 1497-1500, for example. The term "gene" used herein does not only comprise genes of the prokaryote genome but also genes on extra-genomic elements, e.g. plasmids, *etc.*

In an even more preferred embodiment of the conjugate according to the invention, the peptide-nucleic acid (PNA) is directed against a gene which gives

an antibiotic resistance, preferably the antibiotic resistance is a resistance to penicillin, ampicillin, kanamycin or tetracycline.

Furthermore, the conjugate may optionally contain a spacer which is located between the transport mediator and the compound to be transported, e.g. the peptide-nucleic acid (PNA). The spacer serves for eliminating or favorably influencing optionally existing steric interactions between the components.

The structure of the conjugate according to the invention is preferably as follows: transport mediator-spacer-compound to be introduced. The spacers polylysine, polyglycine or poly(glycine/lysine) are particularly preferred. The length of the spacer is preferably within a range of 2 to 6 amino acids for the purposes according to the invention. The spacer is preferably linked with the transport mediator by means of a cleavable disulfide bridge (-S∩S-).

In a particularly preferred embodiment of the conjugate according to the invention, the peptide-nucleic acid (PNA) comprises the following sequence: ATTGTTAGATTTCAT (orientation: N-terminus/sequence/C-terminus).

The present invention finally also relates to a medicament comprising a conjugate according to the invention, optionally together with a suitable carrier. The medicament preferably comprises an antibiotic for which the prokaryote was sensitized by administering the conjugate. Suitable carriers and the formulation of such medicaments are known to the person skilled in the art. Suitable carriers are e.g. phosphate-buffered common salt solutions, water, emulsions, e.g. oil/water emulsions, wetting agents, sterile solutions, *etc.* The medicaments are preferably administered parenterally, transdermally or subcutaneously. The suitable dosage is determined by the attending physician and depends on various factors, e.g. on the patient' age, sex and weight, the kind and stage of the infection, the kind of administration, *etc.*

Finally, the present invention relates to the use of a conjugate according to the invention for treating a prokaryotic infection, this infection being preferably caused by a prokaryote which is resistant to an antibiotic.

Legends of the figures:

Figure 1: Diagram of a conjugate according to the invention using defensin as a transport mediator and a PNA directed against a resistance to ampicillin or kanamycin

Figure 2: Presentation of a pDNA segment of plasmid pBR322 including the sequences used for the design of a PNA conjugate

The sequence of the anti-gene PNA directed against an ampicillin resistance is shown in the figure below. The underlined region of the beta-lactamase-encoding pDNA sequence of pBR322 corresponds to the target region for the PNA conjugate.

Figure 3: List of Holin-protein sequences which are suited as a transport mediator in the conjugates according to the invention

The amino acid sequences of the 28 individual holins are shown in the one-letter code.

Figure 4: Results of treating the competent *E. coli* resistant to ampicillin by transformation with pBR322 and/or to kanamycin by transformation with pEGFP-N_1 (Clontech, Germany, Heidelberg) with or without (control) the conjugates according to the invention

Top row: kanamycin culture medium + kanamycin resistance conjugate (500 nM); bottom row: ampicillin agar + ampicillin resistance conjugate (500 nM); left column: controls (without PNA conjugates). A clear effect of the conjugates according to the invention (regaining the antibiotic sensitivity) can be observed.

Figure 5: Results of the treatment of intact (non-competent) *E. coli* resistant to kanamycin with or without (control) the conjugates according to the invention

Top row: kanamycin culture medium + kanamycin resistance conjugate (2 μM); bottom row: kanamycin culture medium + kanamycin resistance conjugate (20 μM); left columns: controls (without PNA conjugates). A clear effect of the conjugates according to the invention (regaining the antibiotic sensitivity) can

already be observed at a concentration of 2 μM. All of the bacteria are again sensitized, *i.e.* killed, at 20 μM.

Figure 6: Results of the treatment of intact (non-competent) *E. coli* resistant to kanamycin at different concentrations of the conjugates according to the invention to determine the optimum bacterial concentration for the purpose quantification

Kanamycin culture mediums were used onto which resistant bacteria were plated. In order to determine the optimum bacterial concentration, a dilution series having the kanamycin resistance conjugate were used with decreasing decimal powers.

Figure 7: Results of treating intact (non-competent) *E. coli* resistant to kanamycin or ampicillin with the conjugates according to the invention

Plate K1: control, plate 2: 250 nM kanamycin-resistance conjugate; plate 3: 250 nM ampicillin resistance conjugate. The results show a bacterial inhibition for ampicillin and kanamycin with 250 nM conjugate.

Figure 8: Results of treating intact (non-competent) *E. coli* resistant to ampicillin with the conjugates according to the invention

as in figure 7; K2: control, plate 4: 250 nM ampicillin resistance conjugate

Figure 9: Results of treating intact (non-competent) *E. coli* resistant to kanamycin with the conjugates according to the invention

as in figure 6; K1: control; plate 1: 2.5 μM kanamycin resistance conjugate; plate 2: 250 nM kanamycin resistance conjugate.

Figure 10: Structure of defensin used for the conjugates according to the invention following cyclization (top) and following the formation of three disulfide bridges (left bottom)

The hypothetical spatial conformation is shown on the right bottom.

Figure 11: Results of treating HeLa cells with a conjugate according to the invention

A: untreated control

B: cells treated with the conjugate according to the invention

The invention is further described by means of the below examples.

Example 1
General Methods

(A) Cell culture

The bacteria were plated onto agar with LB broth (and the corresponding antibiotics) and incubated at 37°C overnight. HeLa cells were cultured in a liquid culture under common conditions.

(B) PNA Synthesis

Peptide nucleic acid (PNA) imitates a DNA and was originally developed as a reagent for the sequence-specific recognition of double-stranded DNA via a conventional triple helix formation. For the solid phase synthesis the Fmoc strategy was used by means of a fully automated synthesis device (Syro II, Multisyntech, Witten, Germany). The synthesis was carried out on a 0.05 mmol Fmoc-AS polystyrene resin (1% cross-linked). The coupling reagent used was 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU). The side chain-protecting groups were Lys(Boc), Asp(OBut), Ser(But), Cys(Trt) and Asn(Trt). The protected peptidyl resin was treated with 20 % piperidine in dimethylformamide. The cleavage and separation of the protecting groups were obtained by treatment with 90 % trifluoroacetic acid, 5 % ethane dithiol, 2.5 % thioanisole and 2.5 % phenol (v/v/v) at room temperature for 2.5 hours. All of the products were precipitated in ether and purified by preparative HPLC (Shimazu LC-8A, Shimazu, Duisburg, Germany) on a YMC ODS-A 7A S-7 µm reverse phase HPLC column (20 x 250 mm) using 0.1 % trifluoroacetic acid in water (A) and 60 % acetonitrile in water (B) as an eluting agent. The peptides were eluted with a linear gradient of 25 % B to 60 % B at a flow rate of 10 ml/min within 40 minutes. The fractions corresponding to the purified conjugate were lyophilized. Sequences of individual molecules and the complete bimodular construct were characterized by analytical HPLC (Shimadzu LC-10) and laser desorption mass

spectroscopy (Finnigan Vision 2000, Finnigan MAT, San Jose, CA, U.S.A.) as described below.

The sequence of the PNA directed against an ampicillin resistance was as follows: H_2N-ATTGTTAGATTTCAT-COOH. This is a sequence which can hybridize with the region of position 86 to position 100 of the pDNA of pBR322 (GeneBank accession number J01749). The sequence of the PNA used against the kanamycin resistance was H_2N-TCTTGTTCAATCAT-COOH.

(C) Chemical synthesis of defensin

For the solid phase synthesis of defensin (*cf.* figure 10 as regards the amino acid sequence and structure) the Fmoc strategy (Merrifield, J. Amer. Chem. Soc. 85 (1963), 2149-2154; Ruegg and Rudinger, Methods Enzymol. 47 (1977), 111-126) was used with a fully automated synthesis device (ABI 431; Applied Biosystems, Germany, Darmstadt). The synthesis was carried out on a 0.05 mmol Fmoc-Arg(Pbf) polystyrene resin (1% cross-linked). 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU) was used as the coupling reagent. The side chain-protecting groups were: Thr(But), Arg(Pbf). Three different selectively cleavable protecting groups were used for Cys. t-Butylthio was used for Cys(3,15), acetamidomethyl was used for Cys(5,13) and a trityl group was employed for Cys(7,11).

In the first step, the t-butylthio protecting group was cleaved using tris(2-carboxyethyl)phosphine (TCEP) and the sulfur bridge was cleaved with 20 % DMSO in water. In the second step, the acetamidomethyl protecting group was cleaved and at the same time, the second sulfur bridge was oxidized with a 0.01 mole iodine solution. The protected peptidyl resin was treated with 20 % piperidine in formamide for 12 min. and then thoroughly washed with dimethylformamide. The protecting groups were cleaved and removed from the peptide resin by treatment with 90 % trifluoroacetic acid, 5 % ethane dithiol, 2.5 % thioisanole and 2.5 % phenol (v/v/v/v) at room temperature for 2.5 hours. The product was precipitated in ether. The raw material was purified on preparative HPLC (Shimazu LC-8A, Shimazu, Duisburg, Germany) on a YMC-Pack ODS-A,S-5 μm reverse phase HPLC column (20 x 150 mm) using 0.1 % trifluoroacetic

acid in water (A) and 60 % acetonitrile in water (B) as an eluting agent. The peptide was eluted with a linear gradient of 25 % B to 60 % B at a flow rate of 20 ml/min for 40 min. The fractions corresponding to the purified peptide were lyophilized.

As the last step, a head/tail/cyclization was carried out with propane phosphonic acid anhydride (T3P), and the purification method was repeated. The purified material was characterized by analytical HPLC (Shimadzu LC-10) and laser desorption mass spectroscopy (Finnigan Vision 2000, Finnigan MAT, San Jose, CA, U.S.A.).

Peptide purification:
Gradient: analytical 5%→80% (in a period of 35 min.); preparative: 5%→80% (in a period of 40 min.);
Purity: >90%.

(B) Linkage reactions

The linkage reactions were effected as described in German patent application no. 199 33 492.7 under mild oxidative conditions (DMSO/H_2O). For this purpose, cysteine groups of defensin and the spacer H-S-**Gly** and the PNA were oxidized in a region of 2 mg/ml in a 20 % DMSO/water solution. The reaction was complete after about 5 hours. The course of the oxidation was monitored by analytical C18 reverse phase HPLC (Tam *et al.*, J. Amer. Chem. Soc. 113 (1991)). The components were linked according to the Merrifield method (Merrifield, J. Americ. Chem. Soc. 85 (1963), 2149).

The thus synthesized PNA module has the following structure:
$DEFENSIN_{human}$-S∩S-$(Gly)_X$-$PNA_{AmpR/NeoR}$
X = 1-5

The purification was made by means of reverse phase HPLC, followed by lyophilization. Having determined the mass by means of MS, the lyophilizate was dissolved in a defined volume of physiological common salt solution to give a stock solution of 10 μM.

Example 2

Determining the activity of the conjugates according to the invention in the case of antibiotic-resistant *E. coli* strains

Resistant bacteria were confluently plated onto common agar plates with and without antibiotic and partially treated with the conjugates according to the invention. In a preliminary experiment, already competent *E. coli* (*i.e.* having already perforated membranes) were used; see figure 4; top row: kanamycin culture medium + kanamycin resistance conjugate (500 nM); bottom row: ampicillin agar + ampicillin resistance conjugate (500 nM); left columns: controls. A marked effect of the conjugates according to the invention (regaining the antibiotic sensitivity) can be observed.

In the then following experiments (shown in figures 5 to 9), intact (non-competent) *E. coli* were used, and the conjugates directed against an antibiotic resistance were tested at various dilutions. As to the employed bacteria, antibiotics and concentrations, reference is made to the legend of the figures. In any case, the results of these studies clearly show that the conjugates according to the invention effect another sensitization of the bacterium for the corresponding antibiotic by means of a PNA directed against a gene giving antibiotic resistance and that they can thus be combated by means of this antibiotic again.

The non-toxic effect of this conjugate on eukaryotic cells (HeLa cells) was detected by incubation of the cells with/without conjugate (at concentrations as for the experiments with bacteria); see figures 11A+11B.

Claims

1. A conjugate suited for treating prokaryotic infections and comprising the following components:
   (a) a transport mediator penetrating the prokaryotic cell membrane; and
   (b) a compound to be introduced into the prokaryote and directed thereagainst.

2. The conjugate according to claim 1, wherein the prokaryote is a bacterium.

3. The conjugate according to claim 2, wherein the bacterium is a bacterium pathogenic for humans.

4. The conjugate according to any of claims 1 to 3, wherein the transport mediator is a peptide or protein which can penetrate the prokaryotic cell membrane.

5. The conjugate according to any of claims 1 to 4, wherein the transport mediator comprises a phage-holin protein comprising one of the amino acid sequences shown in figure 3 or a fragment or variant thereof, which can penetrate the prokaryotic cell membrane.

6. The conjugate according to any of claims 1 to 4, wherein the transport mediator comprises a defensin.

7. The conjugate according to any of claims 1 to 6, wherein the compound to be introduced is a peptide nucleic acid (PNA) directed against the expression of a gene.

8. The conjugate according to claim 7, wherein the peptide nucleic acid (PNA) is directed against a gene giving antibiotic resistance.

9. The conjugate according to claim 8, wherein the antibiotic resistance is a resistance to penicillin, ampicillin, kanamycin or tetracycline.

10. The conjugate according to any of claims 1 to 9, which has the following structure: transport mediator-spacer-compound to be introduced.

11. The conjugate or conjugate mixture according to claim 10, wherein the spacer is polylysine, polyglycine or poly(glycine/lysine).

12. The conjugate according to claim 10 or 11, wherein the spacer is linked to the transport mediator via a cleavable disulfide bridge.

13. The conjugate according to any of claims 7 to 12, wherein the peptide nucleic acid comprises the sequence H_2N-ATTGTTAGATTTCAT-COOH.

14. A medicament containing a conjugate according to any of claims 1 to 13.

15. The medicament according to claim 14, further containing at least one antibiotic for which the prokaryote was re-sensitized by administering the conjugate.

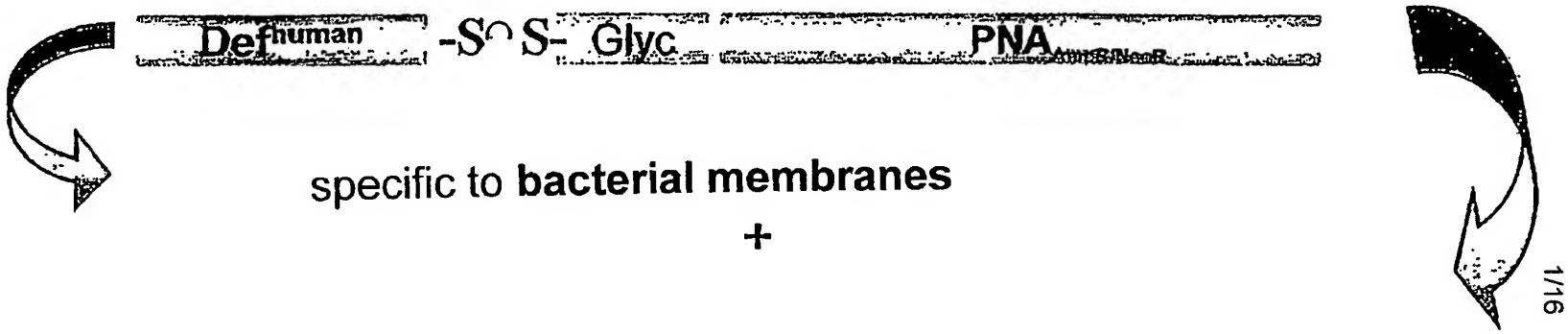
16. Use of a conjugate according to any of claims 1 to 13 or the composition defined in claim 15 for treating a prokaryotic infection.

17. Use according to claim 16, wherein the prokaryotic infection is caused by a prokaryote which is resistant to at least one antibiotic.

Abstract of the Disclosure

The invention relates to a conjugate for treating prokaryotic infections from a transport mediator penetrating the prokaryotic cell membrane and a desired compound to be introduced into the prokaryote and directed thereagainst, which compound is preferably a peptide nucleic acid (PNA) directed against a gene of the prokaryote giving antibiotic resistance.

Strategies:



specific to **bacterial membranes**

+

anti-gene strategy directed specifically against **bacterial genes**

→ combined 'dual strategy'

Fig. 1

Genome site of action – anti-gene PNA

DEFINITION Cloning vector pBR322, complete genome.
ACCESSION J01749 – Genbank
KEYWORDS ampicillin resistance; beta-lactamase; cloning vector; drug resistance protein; origin of replication; plasmid;
SOURCE Cloning vector pBR322.
ORGANISM Cloning vector pBR322.
artificial sequence; vectors.

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TTCTCATGTT TGACAGCTTA TCATCGATAA GCTTTAATGC GGTAGTTTAT CACAGTTAAA      60

TTGCTAACGC AGTCAGGCAC CGTGTATGAA ATCTAACAAT GCGCTCATCG TCATCCTCGG     120

CACCGTCACC CTGGATGCTG TAGGCATAGG CTTGGTTATG CCGGTACTGC CGGGCCTCTT     180
GCGGGATATC GTCCATTCCG ACAGCATCGC CAGTCACTAT GGCGTGCTGC TAGCGCTATA     240
TGCGTTGATG CAATTTCTAT GCGCACCCGT TCTCGGAGCA CTGTCCGACC GCTTTGGCCG     300
CCGCCCAGTC CTGCTCGCTT CGCTACTTGG AGCCACTATC GACTACGCGA TCATGGCGAC     360
CACACCCGTC CTGTGGATCC TCTACGCCGG ACGCATCGTG GCCGGCATCA CCGGCGCCAC     420
AGGTGCGGTT GCTGGCGCCT ATATCGCCGA CATCACCGAT GGGGAAGATC GGGCTCGCCA     480
CTTCGGGCTC ATGAGCGCTT GTTTCGGCGT GGGTATGGTG GCAGGCCCCG TGGCCGGGGG     540
ACTGTTGGGC GCCATCTCCT TGCATGCACC ATTCCTTGCG GCGGCGGTGC TCAACGGCCT     600
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anti-gene PNA

HOOC–TAC TTT AGA TTG TTA–NH_2

Fig. 2

Alignments Holin-Protein (Phagen) - *Transportprotein*

product="probable holin" (GMSE-1)
[Endosymbiont bacteriophage may influence susceptibility to trypanosome infection in tsetse, Dale and Young]
protein_id="AAG50251.1"
db_xref="GI:12276078"
translation="MPCLIHLVGWGSSPGSALIREQAIGAGLAAWMTCLRGRYLGRGWRKTTFDAAICALIAWF
ARDGLALVGIDNQFSYLSSIIVGYLGNDYLGALLRRRLEKKS GESNAPQ

product="holin protein" (Listeria innocua)
protein_id="CAA61518.1"
translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG
SLATMIWAGALAGAGGTGLFEQFTNRAKKYGKDD

product="holin" (bacteriophage 80 alpha)
specific_host="Staphylococcus aureus RN450
function="makes hole in membrane"
protein_id="AAB39698.1"
db_xref="GI:1763242"
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT
DPTSKGVSDSSIAQTYQAPRDSKKEEQQVTWKSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN
EYHHGGGDNASKIN

product="holin" (Staphylococcus bacteriophage phi 11)
note="ORF3; structural homologue of holin"
protein_id="AAA99522.1"
db_xref="GI:511841"
translation="MDINWKLRFKNKAVLTGLVGALFVFIKQVTDLFGLDLSTQLNQASAIIGAILTLLTGIGVIT
DPTSKGVSDSSIAQTYQAPRDSKKEEQQVTWKSSQDSSLTPELSAKAPKEYDTSQPFTDASNDVGFDVN
EYHHGGGDNASKIN

product="putative holin 1" (Streptococcus pneumoniae bacteriophage MM1)
function="lysis protein"
protein_id="CAC48114.1"
db_xref="GI:15074937"
translation="MKIEFFNFLRSVIQTEDGLVLYALALIVSMEIIDFVTGTIAAIINPDIEYKSKIGINGLLRKISGV
LLLMILIPASVLLPEKTGFAFLYSICLGYIAFTFQSLIENYRKLKGNVTLFQPIVKVFQRLLEKDDDTKKGE

gene="orf87a" (Streptococcus thermophilus bacteriophage Sfi21)
product="holin"
protein_id="CAA64941.1"
db_xref="GI:2292749"
translation="MKKRKKKMINFKLRLQNKATLVALISAVFLMLQQFGLHVPNNIQGINTLVGILVILGIITDP
TTKGIADSERALSYIQPLDDKEVY

gene="hol500" (Bacteriophage A500);(Listeria monocytogenes)
protein_id="CAA59363.1"
/db_xref="GI:853745"
/translation="MMKMEFGKELLVYMTFLVVVTPVFVQAIKKTELIPSKWLPTVSILVGAILGALATSLDGSG
SLATMIWAGALAGAGGTGLFEQFTNRAKKYGKDDK

product="holin" (Bacteriophage PL-1)
protein_id="BAA96748.1"
translation="MQNELLQVLAIAFVIAPITTGFTEIFKRYTPAEGKLLPVLSIGTG
ILLACVWAMAFGHLPLIGAYALAGMLSGLASVGVYQIVKPNEEVK

Fig. 3(1)

gene="lydA" (Bacteriophage P1) (enterobacteriae)
codon_start=1
product="holin"
protein_id="CAA61014.1"
db_xref="GI:974764"
translation="MLDTQELAPVAIALLLSVIGGIGTFLMDVRDGRQSGNLLGLVTEIFVAVTAGAVAYLLGQH
EGWELSITYLMVTIASNNGHEVISGMKRVNIDSILNVLTSL VKKGGGK

gene="S" (Bacteriophage H-19B)
note="similar to Bacteriophage 21 lysis gene S, encoded by GenBank Accession Number M65239" /
product="putative holin protein"
protein_id="AAD04658.1"
db_xref="GI:2668771"
translation="MEKITTGVSYTTSAVGTGYWLLQLLDKVSPSQWVAIGVLGSLLFGLLTYLTNLYFKIREDR
RKAVRGE

gene="hol" (Bacteriophage A118)
function="forms unspecific lesions into cytoplasmic membrane prior to lysis"
specific_host="Listeria monocytogenes"
note="ORF24; two products may be translated from this gene (hol-96 and hol-93)"
product="holin"
protein_id="CAB53810.1"
db_xref="GI:5823622"
translation="MIEMEFGKELLVYMTFLVVVTPVFVQAIKKTELVPSKWLPTVSILIGAILGALATFLDGSGS
LATMIWAGALAGAGGTGLFEQFTNRSKKYGEDDK

gene="Hol" (Lactobacillus casei bacteriophage A2)
product="putative holine"
protein_id="CAB87385.1"
db_xref="GI:7573220"
translation="MKINWKVAVLSVKFWLALVPAALLVVQTAAAVFGYNWDFANLGKELTAVINAVFALLTI
VGVAVDPTTEGVSDSQQALAYPALITTKAAKIKSLEDQIKALQADKAADQATSAASEVVPETSSAAPAE
SAPESVAPVASEEVK

gene="Hol" (Lactobacillus bacteriophage phig1e)
product="holin"
protein_id="CAA66751.1"
db_xref="GI:1926366"
translation="MDIITSLNLATAGELALISFFIGVIVQAIKKTGKVKNTYLPFISMGIGILAGLAAVVVTKDTN
YLNGAVAGLIVGAATSGLTDGLSVGTSAVTTAKATKDAAKTAAITQAVLNSINTTKSSDTTQVANTSN
TEGGSTSETQK

product="holin" (Lactobacillus delbrueckii subsp. lactis bacteriophage LL-H)
protein_id="AAC00556.1"
db_xref="GI:623083"
translation="MTLIDWFNLIVAIGTIALAVVASVYVHLKAKIDTKTAAGKAFDLVGKLAVWAVNEAEHSQ
DGGAAKREFAAKLISDQLKAKGITGIDEKMVYGAVETAWKEA IENVK

product="holin protein" (Lactococcus phage c2)
protein_id="AAD20611.1"
db_xref="GI:4426933"
translation="MIETLRAIGLVVFMQLLSLALEFIDTGTLKPSVRKRIAVELMVL

gene="hol" (bacteriophage phiAM2)
note="hydrophobic pore-forming protein"
product="holin"
protein_id="AAG24367.1"
db_xref="GI:10880732"
translation="MFFNNKFYNVIKWAVLTALPALSVFIGVIGKAYGWGGTDLAIITLNAFTVFLGTLAGVSAV
KYNSQPNDTKENK

Fig. 3(2)

product="holin"
protein_id="AAG24367.1"
translation="MFFNNKFYNVIKWAVLTALPALSVFIGVIGKAYGWGGTDLAIITLNAFTVFLGTLAGVSAV
KYNSQPNDTKENK

product="holin" (Bacteriophage Tuc2009)
protein_id="AAA32614.1"
db_xref="GI:496282"
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGAPFGYKWDFVILNQQLAAVVNAAFALLAI
VGVVADPTTSGLGDSDRVLNKDKSEENK

product="holin" (Bacteriophage TPW22)
function="formation of non-specific lesions in the cytoplasmic membrane"
protein_id="AAF12704.1"
db_xref="GI:6465904"
translation="MNQINWKLRLKSKAFWLALLPALFLLIQAIGASFGYKWNFVILNQQLAAVVNAAFALLAI
VGVVADPTTSGLGDSDRVLNKDKSEENK

product="holin" (homology to Orf78 of phage HP1 and gene S of phage P21)
protein_id="AAC45168.1"
db_xref="GI:915370"
translation="MRFNMLKNSETTGAYVGSAIAIYSGFTLADWAAIFGILFGLFT M LINWYYKNK
EIKLKETALKQKIDLKEGDHE

product="holin" (Bacillus phage GA-1)
function="host cell lysis, holin formation"
protein_id="CAC21535.1"
db_xref="GI:12141291"
translation="MFEFFHSLMETDDTKVYFLLGIIGVLNIVDFFFGFINAKFNKSIAYKSSKTIDGIMRKMKFTI
MAILFIPVSVLMPEPIGLGALYVFYFGYIYAELNSILSH LKLSEDGKETEVFLDFINTFFNSTKGDKKDD

gene="hol187" (Staphylococcus phage 187)
function="forms pores to allow access of lysin to CW"
product="holin protein Hol187"
protein_id="CAA69023.1"
db_xref="GI:2764984"
translation="MLMVIMVGNVGIYLTIFLIDTGTLRHQATQEIWHGIDILKGLKC LETLLILSLNQVI

gene="s" /function="holin" (Shigella dysenteriae)
product="S protein"
protein_id="CAC05628.1"
db_xref="GI:9955825"
translation="MYQMEKITTGVSYTTSAVGMGYWFLQFLDRVSPSQWAAIGVLGSLLFGLLTYLTNLYFKI
REDRRKAARGE

gene="E"
protein_id="CAA42879.1"
db_xref="GI:14781"
db_xref="SWISS-PROT:P31280"
translation="MERWTLLDILAFLLLLSLLLPSLLIMFIPSMYKQHASLWKARSLAKTLSMASSARLTPLSSS
RTPCVLKQDSKKL

gene="xhlB" (B.subtilis DNA (28 kb PBSX/skin element region)
product="holin-like protein"
protein_id="CAA94048.1"
db_xref="GI:1225964"
db_xref="SWISS-PROT:Q99163"
translation="MNTFDKGTVIRTVLLLIALINQTMLMLGKSPLDIQEEQVNQLADALYSAGSIAFTIGTTLAA
WFKNNYVTEKGKKQRDLLRDNNLTK

Fig. 3(3)

gene="bhlA" (Bacillus subtilis 168 prophage)
product="holin-like protein"
protein_id="AAC38301.1"
db_xref="GI:2997596"
translation="MEMDITQYLSTQGPFAVLFCWLLFYVMKTSKERESKLYNQIDSQNEVLGKFSEKYDVVIE
KLDKIEQNFK

gene="bhlB" (Bacillus subtilis 168 prophage)
product="holin-like protein"
protein_id="AAC38302.1"
db_xref="GI:2997597"
translation="MFENIDKGTIVRTLLLAIALLNQIMVMLGKAAFIINEEDINHLYDCLYTIFTIVFTTSTTTAA
WFKNNYITAKGKKQKQVLKKENLFK

gene="hol" (Bacteriophage phi-Ea1h)
specific_host="Erwinia amylovora
function="pore formation"
product="holin"
protein_id="CAC17008.1"
db_xref="GI:11342496"
translation="MRKIYYVIITTIVVAGLIWAFIATQVNTGVTSKRQEDALAVSEANVGIGKEAKDQGEQATK
RADVAKEQRTHQINQLKDKLHEKAESYDSIPLSPSDVDILC RAYRSTDPVCSPTVKSD

Fig. 3(4)

Alignments **Lysis-Protein** (Phagen)

product="lysis protein" (Phage phiX174)
function="host cell lysis"
protein_id="CAA84691.1"
translation="MVRWTLWDTLAFLLLLSLLLPSLLIMFIPSTFKRPVSSWKALNLRKTLLMASSVRLKPLNCS
RLPCVYAQETLTFLLTQKKTCVKNYVQKE

Fig. 3(5)

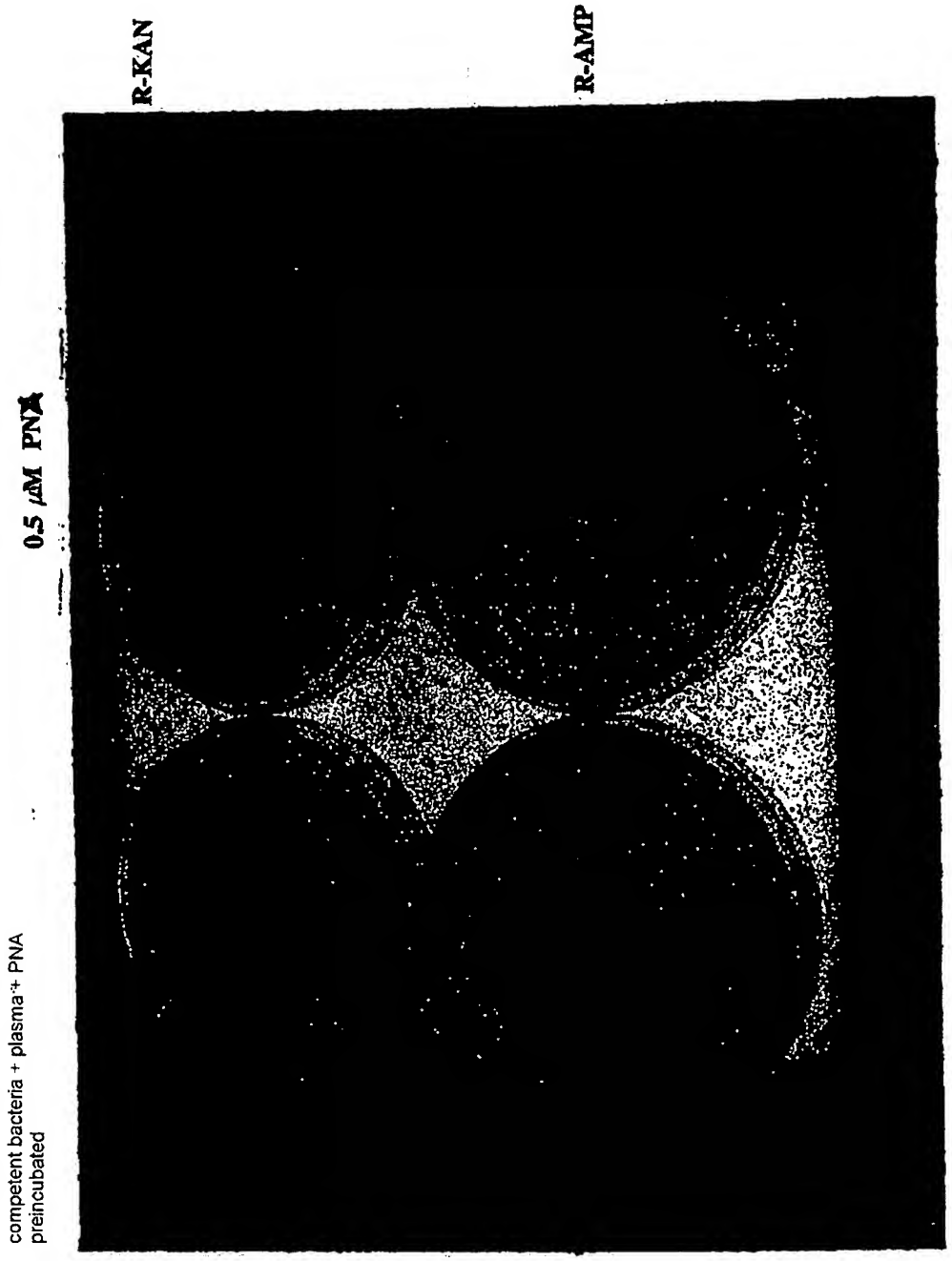

R-KAN
R-AMP
0.5 µM PNA
competent bacteria + plasma + PNA
preincubated

Fig. 4

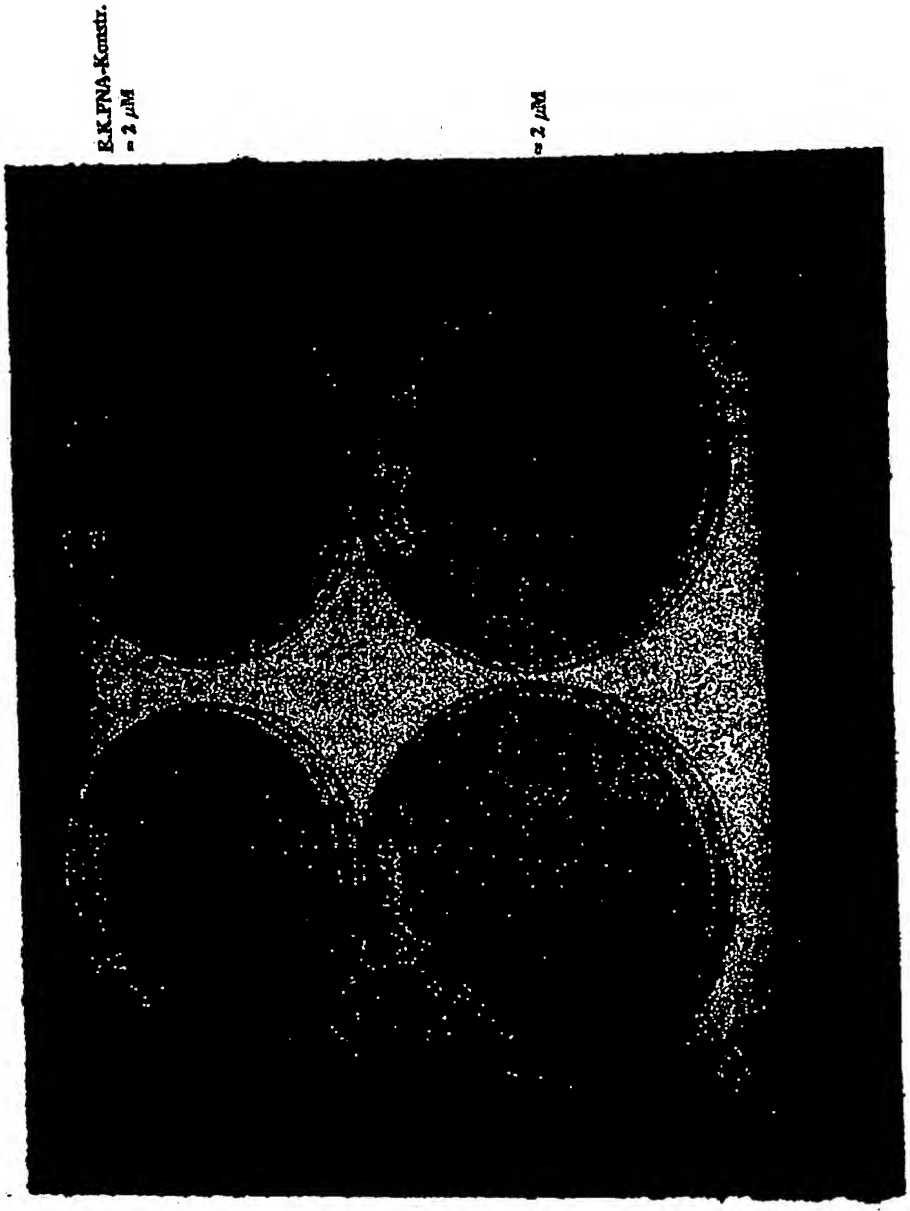

R-K-FNA-Konstr.
= 2 µM
= 2 µM
intact bacteria + incubation with α-Kan-FNA

Fig. 5

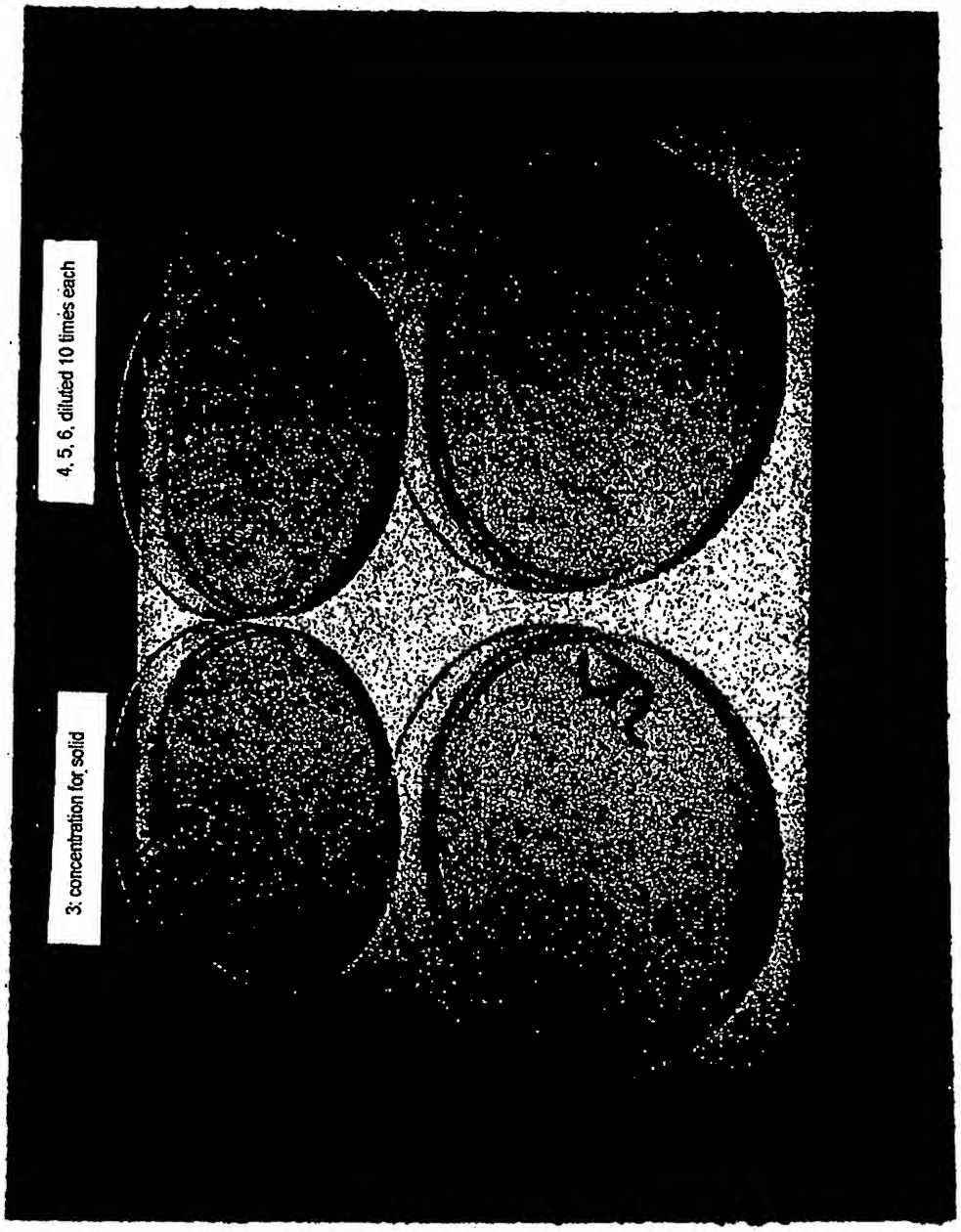


Fig. 6

dilution series bact. (E. coli)
with Kan,R plasmid

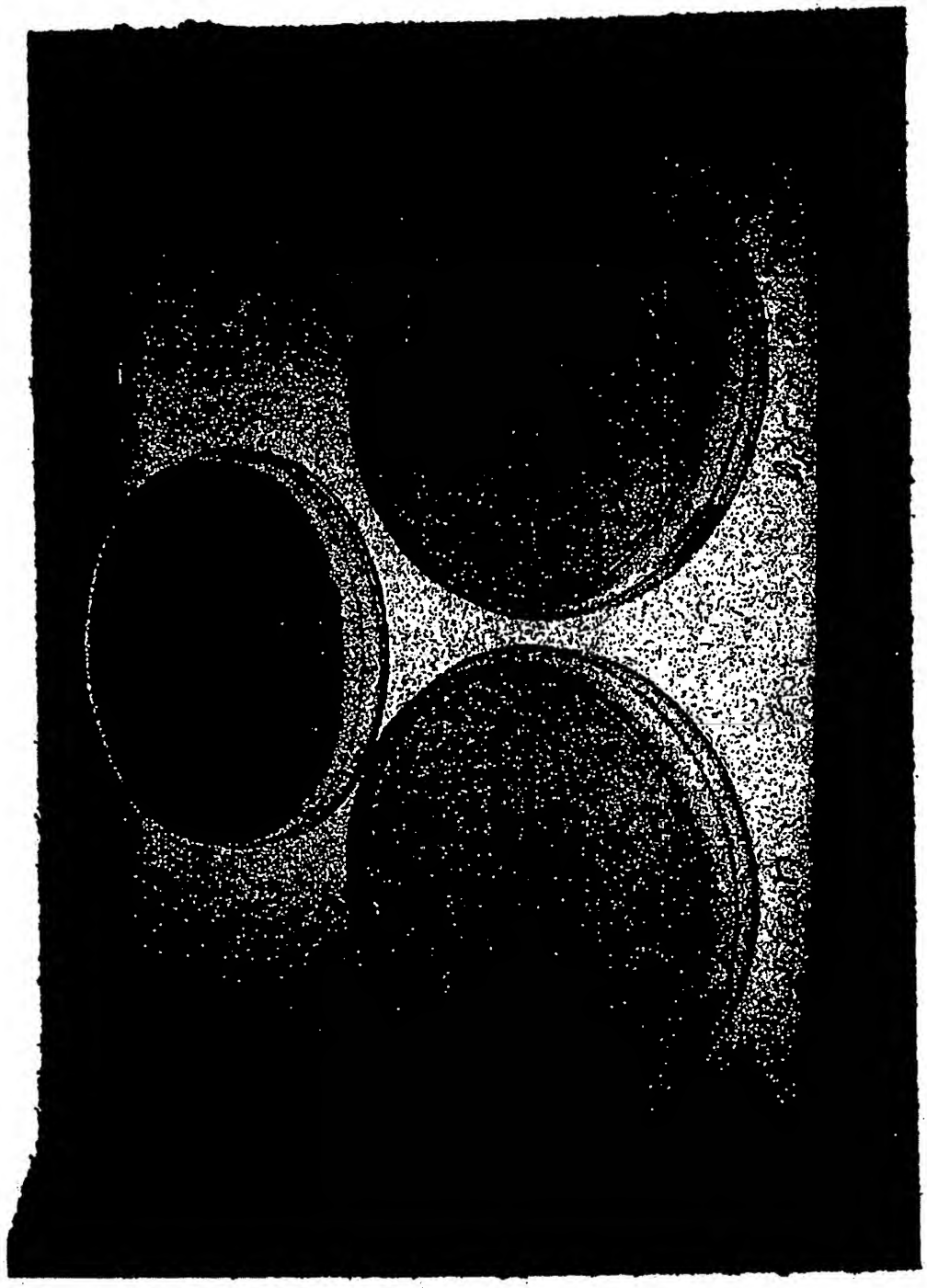


Fig. 7

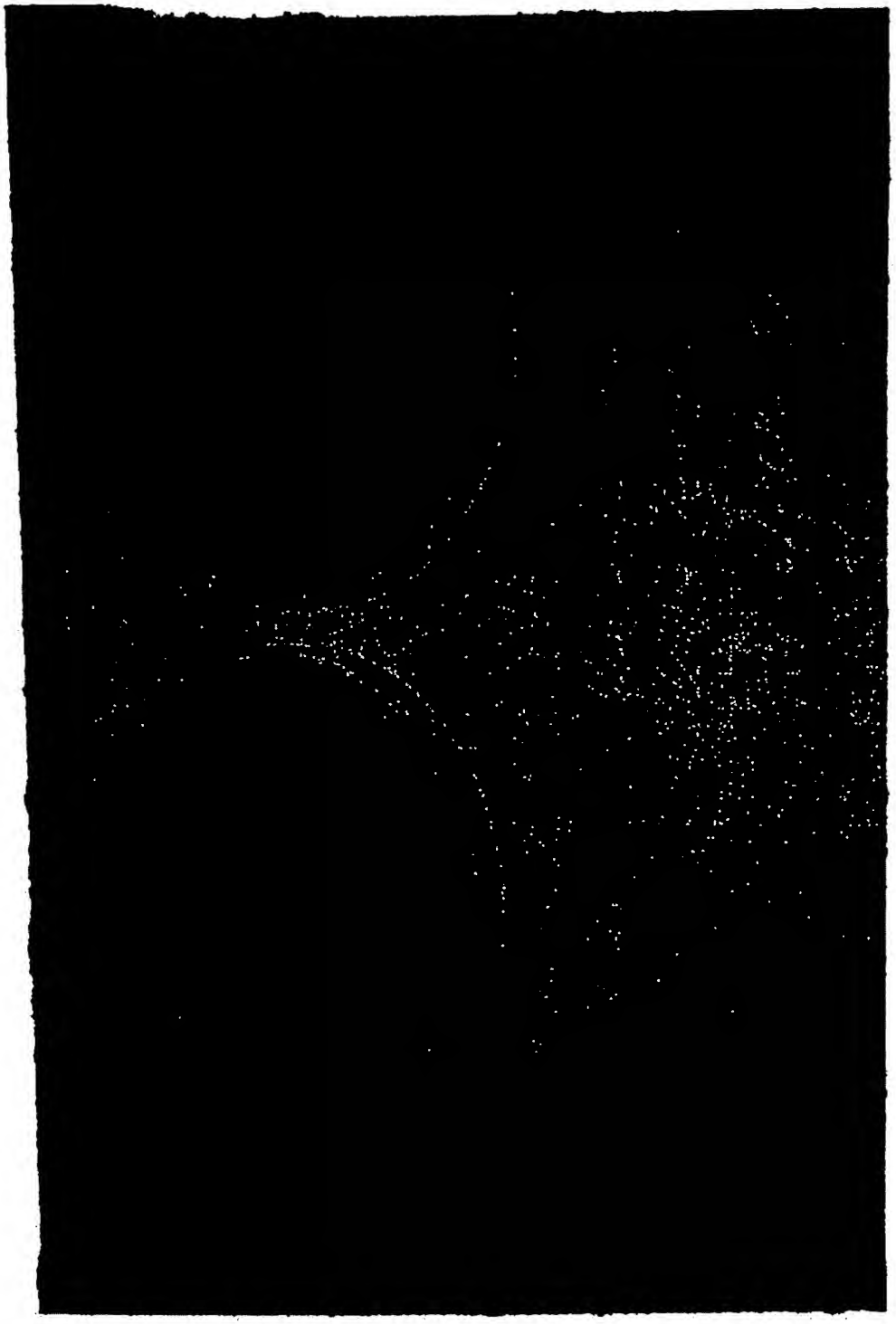


Fig. 8

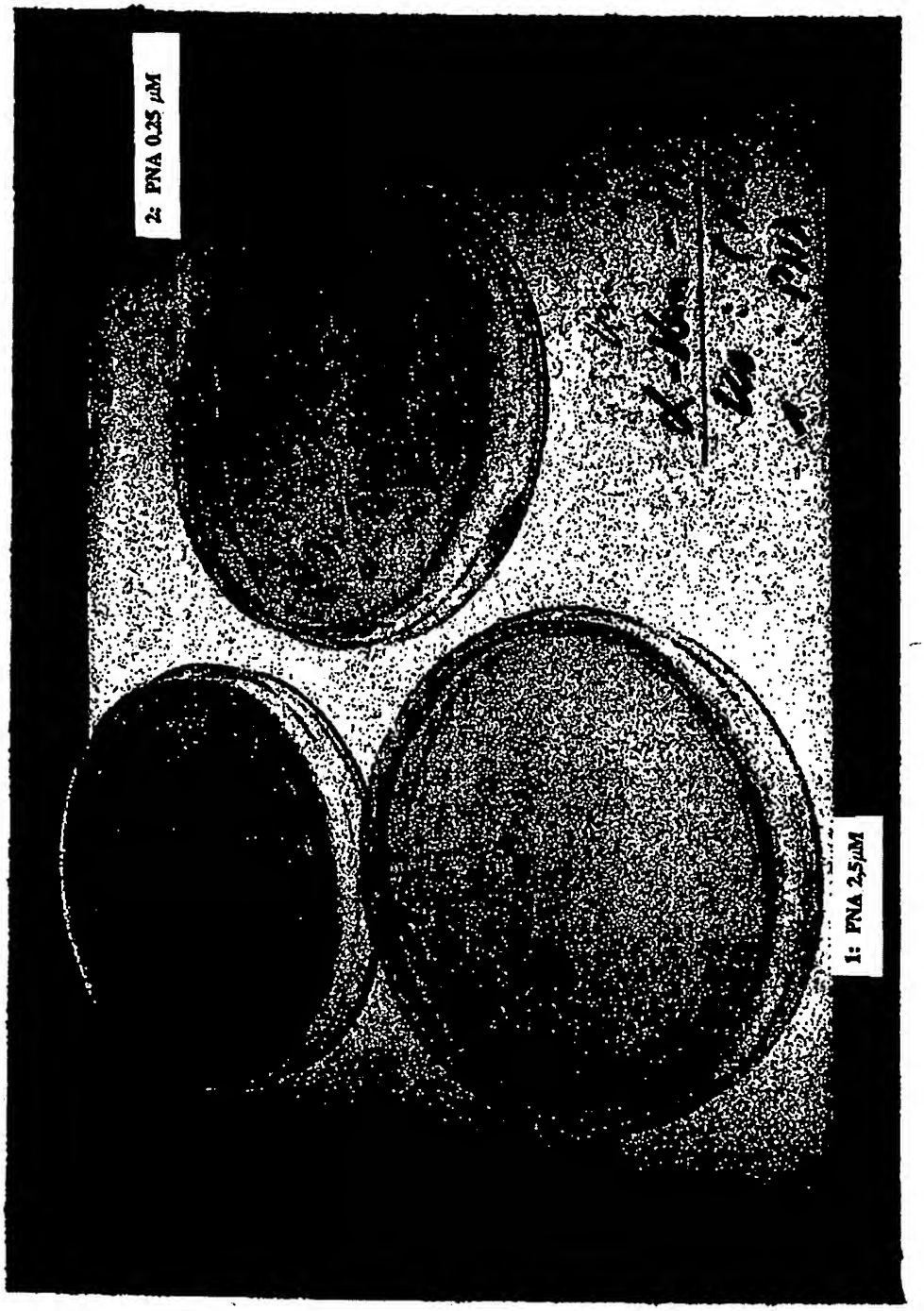
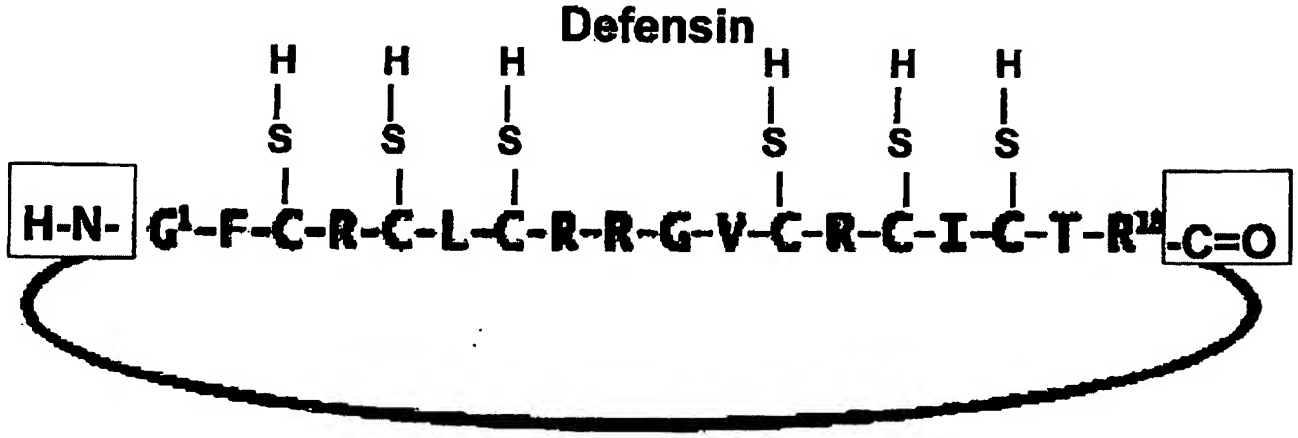


Fig. 9


Defensin
H-N-
G¹-F-C-R-C-L-C-R-R-G-V-C-R-C-I-C-T-R¹⁶
-C=O

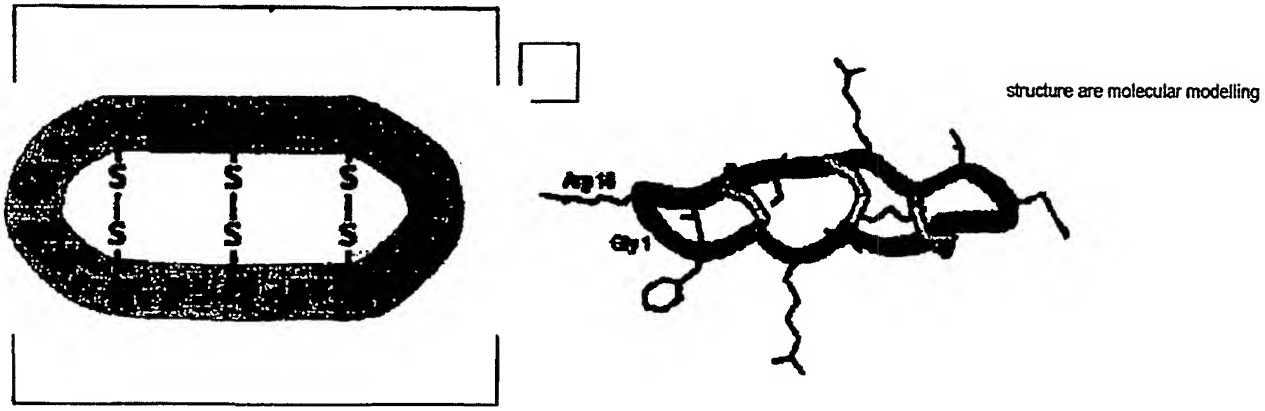

structure are molecular modelling
Arg¹⁶
Gly¹

Fig. 10

Fig. 11(A)

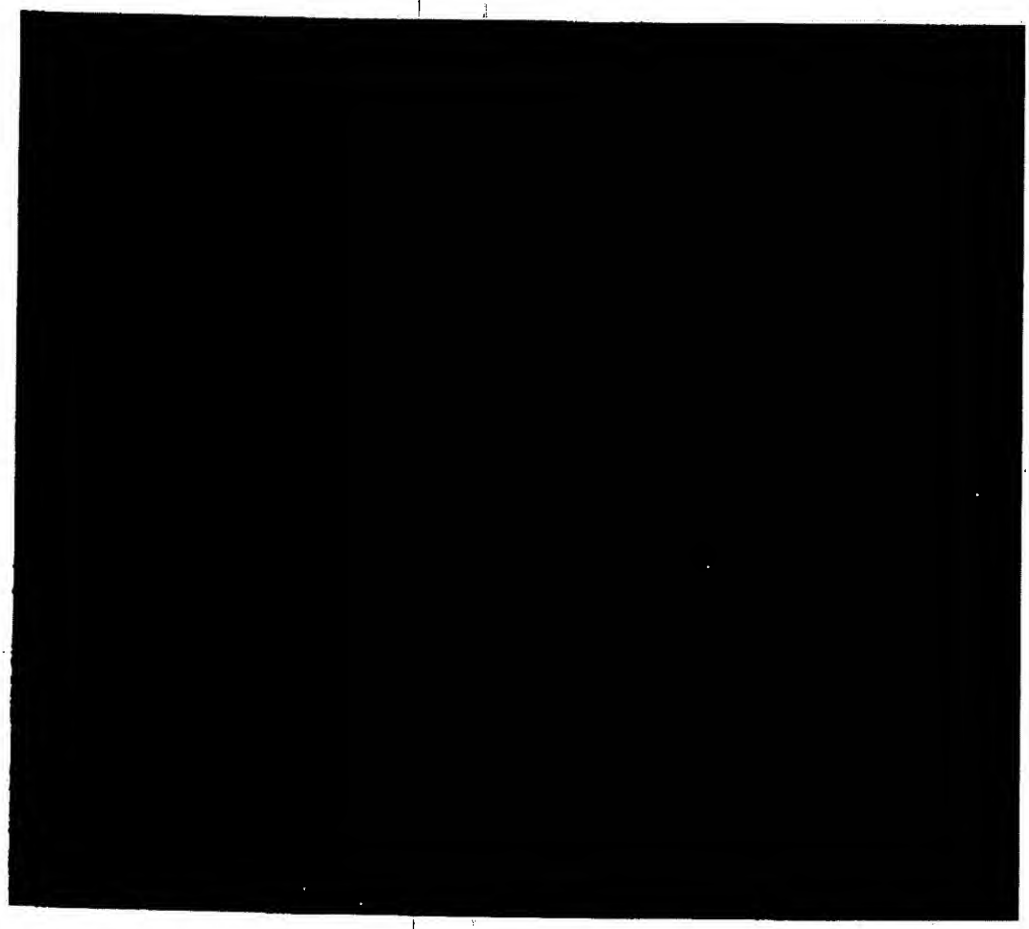


Fig. 11(B)